

Disruption of Amino Acid Metabolism in Human Myelogenous Leukemic Cell Lines Destined to Die after Contact with Metal Plates

HIROYUKI ONUKI¹, HIROSHI SAKAGAMI², TAKASHI YAMAZAKI¹, ATSUSHI YAMAZAKI³,
YASUSHI HIBINO³, YOSHIKO YOKOTE⁴, HIROSHI NAKAJIMA³ and JUN SHIMADA¹

Divisions of ¹Oral and Maxillofacial Surgery and ²Pharmacology, Department of Diagnostic and Therapeutic Sciences,

³Division of Dental Biomaterials Science, Department of Restorative and Biomaterials

Sciences, Meikai University School of Dentistry, Sakado, Saitama 350-0283;

⁴Faculty of Science, Josai University, Saitama 350-0295, Japan

Abstract. *Changes in amino acid metabolism during cell death of human myelogenous leukemic cell lines (HL-1, ML-1, KG-1) induced by contact with gold (Au), silver (Ag) or palladium (Pd) were investigated. All three leukemic cell lines consumed glutamine and serine at the highest rate (amounting to 50%-58% and 12%-16% of the total amino acid consumption, respectively). HL-60 cell growth was slightly stimulated by contact with any metal plate. Contact with Ag or Pd, but not Au plates occasionally induced cytotoxicity against ML-1 and KG-1 cells. In such cases, glutamine consumption was inhibited by 88%-90% and consumption of other amino acids completely ceased. This was accompanied by the enhanced production of arginine, glycine and glutamic acid. These data suggest the tight association of the disruption of amino acid metabolism with the cell death induced in human myelogenous leukemic cell lines by contact with metal plates.*

Dental alloys have been reported to induce allergic reactions in the oral cavity, though infrequently (1), possibly by the stimulated release of metal ions from the alloys (2, 3). The metal ions may be incorporated into the cells, possibly *via* metal transporter-mediated endocytosis (4, 5). Numerous studies have shown cytotoxic activity and tissue-damaging activity of metal extracts (6-8). As far as we know, however, no detailed study of cytotoxicity induced by direct contact with metals has been reported. We recently

developed an assay system to investigate the interaction between metals and cultured cells (9). Using this system, we found that direct contact with a copper (Cu) plate induced non-apoptotic cell death characterized by a smear pattern of DNA fragmentation, minor caspase-3 activation and vacuolization without affecting the cell surface microvilli or mitochondrial integrity in a human promyelocytic leukemic cell line (HL-60) (9). Contact with the Cu plate also induced non-apoptotic cell death accompanied by a rapid decline in glutamine consumption and the enhanced production of glutamic acid and glycine in human gingival fibroblast prepared from periodontal tissues (10). Here, whether the plates of gold (Au), silver (Ag) and palladium (Pd), metals used as constituents in dental alloys, induce cytotoxicity against three human myelogenous leukemic cell lines (HL-60, ML-1, KG-1), and if so, whether any changes in amino acid metabolism are similar were investigated.

Materials and Methods

Materials. The following materials were obtained from the companies indicated: Au, Ag, Pd plates (99.99%, 20x20x0.5 mm) (Tokuriki Honten, Co, Japan); fetal bovine serum (FBS), RPMI-1640 medium (Sigma Chem. Co., St. Louis, MO, USA); trichloroacetic acid (TCA) (Wako Pure Chem Co., Tokyo, Japan).

Polishing of metal plate surfaces. Cu plates were polished using an alumina-water slurry (micropolish, Buehler; 0.05 µm particle size). After polishing, surfaces were examined using a scanning electron microscope (JSM-6360LV, JEOL, Japan) to confirm the consistency of surface smoothness.

Cell culture. HL-60, ML-1 and KG-1 cell lines (supplied by Prof. Ken Takeda, Tokyo University of Science) were incubated as suspension cultures in RPMI-1640 medium supplemented with 10% heat-inactivated FBS in a humidified 5% CO₂ atmosphere.

Cytotoxicity induced by direct contact with metal plates. Five hundred µL of human myelogenous leukemic cells (1x10⁶) were

Correspondence to: Hiroshi Sakagami, Division of Pharmacology, Department of Diagnostic and Therapeutic Sciences, Meikai University School of Dentistry, Sakado, Saitama 350-0283, Japan. Tel: +81 49 2792758, Fax: +81 49 2855171, e-mail: sakagami@dent.meikai.ac.jp

Key Words: Metal plate, leukemic cells, cell death, amino acid metabolism, glutamine, serine, dental metals.

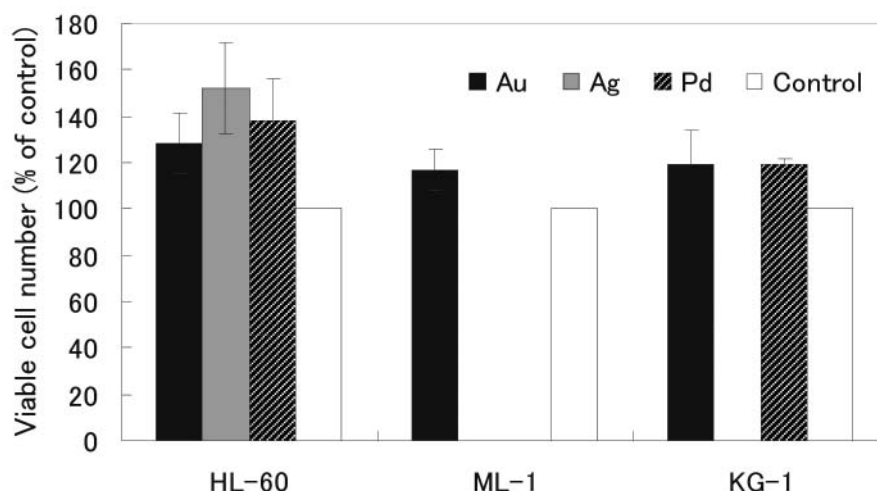


Figure 1. Effect of contact with metal plates on cell viability. Human leukemic cells ($1 \times 10^6/\text{mL}$, 0.5 mL) were inoculated onto an Au, Ag or Pd plate, or plastic plate (control) and the viable cell number was determined after 24 hours, expressed as % of control. The viable number of control HL-60, ML-1 and KG-1 cells at 24 hours was $1.97 \pm 0.26 \times 10^6/\text{mL}$, $1.92 \pm 0.23 \times 10^6/\text{mL}$ and $2.15 \pm 0.66 \times 10^6/\text{mL}$, respectively. Each value represents mean \pm S.D. of three independent experiments.

inoculated onto an Au, Ag or Pd plate (placed in a 3.5-cm dish) and incubated for 0 or 24 hours at 37°C in 5% CO_2 (Figure 1). Cells were recovered from the plates by gentle pipetting and washing with a total of 0.5 mL of phosphate-buffered saline (PBS) without calcium or magnesium (PBS (-)). The viable cell number was determined by cell counting with a hemocytometer after staining with 0.15% trypan blue dye and after correction for the dilution factor of 4 (due to PBS washing and trypan blue addition). The supernatant (medium fraction) obtained after centrifugation at 1,000 rpm for 5 min was used for amino acid analysis.

Determination of free amino acids. Culture supernatant (medium fraction) was mixed with an equal volume of 10% TCA and stood on ice for 30 min. After centrifugation for 5 min at 10,000 xg, the deproteinized supernatant was collected and stored at -40°C . The supernatants (20 μL) were subjected to a JEOL-JLC500/V amino acid analyzer and amino acids were detected using the ninhydrin reaction (9-11). The amino acid concentration was corrected for the dilution factor of 4 (due to PBS wash and TCA addition).

Results

Contact with an Au, Ag and Pd plate for 21 hours induced stimulation of HL-60 cell growth by 28%, 52% and 38%, respectively (Figure 1). Contact with the Au plate stimulated ML-1 cell growth by 17%, whereas contact with the Ag or Pd plate induced cell death in ML-1 cells. Contact with an Au or Pd plate stimulated KG-1 cell growth by 19% respectively, whereas contact with an Ag plate induced cell death (Figure 1).

In HL-60 cells, GLN was consumed at the highest rate (821 nmole/mL/day, amounting to 58.1% of the total amino acid consumption), followed by SER (16.3%), LYS (4.7%),

VAL (4.7%), LEU (3.6%), THR (3.0%), ILE (2.5%) and PHE (2.1%) and MET (1.7%). Changes in the concentration of ARG, ASN, TYR, HIS, CYS and ASP were very small. GLY, GLU and ALA were the major amino acids that were produced during incubation. Contact with an Au, Ag or Pd plate did not induce any apparent change in the consumption or production of any of these amino acids in HL-60 cells (Figure 2A).

In ML-1 cells, GLN was also consumed at the highest rate (960 nmole/mL/day, amounting to 49.8% of the total amino acid consumption), followed by SER (11.5%), LYS (6.2%), Val (6.0%), LEU (5.7%), ILE (4.4%), THR (4.4%), PHE (2.8%), MET (2.4%), ASN (2.3%) and TYR (1.9%) (Figure 2B). Very little change in the concentration of ARG, HIS, GLY, CYS and ASP was observed. GLU and ALA were the major amino acids that were produced during incubation. When ML-1 cells were committed to die after contact with Ag or Pd, the consumption of GLN was reduced by 90.1% and the consumption of SER and other amino acids ceased completely. On the other hand, production of ARG, GLY and GLU was increased by 140, 85 and 173 nmole/mL/day, respectively, whereas the production of ALA was reduced by 179 nmole/mL/day.

In KG-1 cells, GLN was again consumed at the highest rate (1000 nmole/mL/day amounting to 51.2% of the total consumption of amino acids), followed by SER (11.6%), LYS (6.4%), Val (6.1%), LEU (5.8%), ILE (4.3%), THR (4.2%), PHE (2.9%) and MET (2.4%) (Figure 2C). Very little change in the concentration of TYR, ASN, CYS and ASP was observed. GLU and ALA were the major amino acids that were produced during incubation. When KG-1 cells were committed to die after contact with Ag, the consumption of

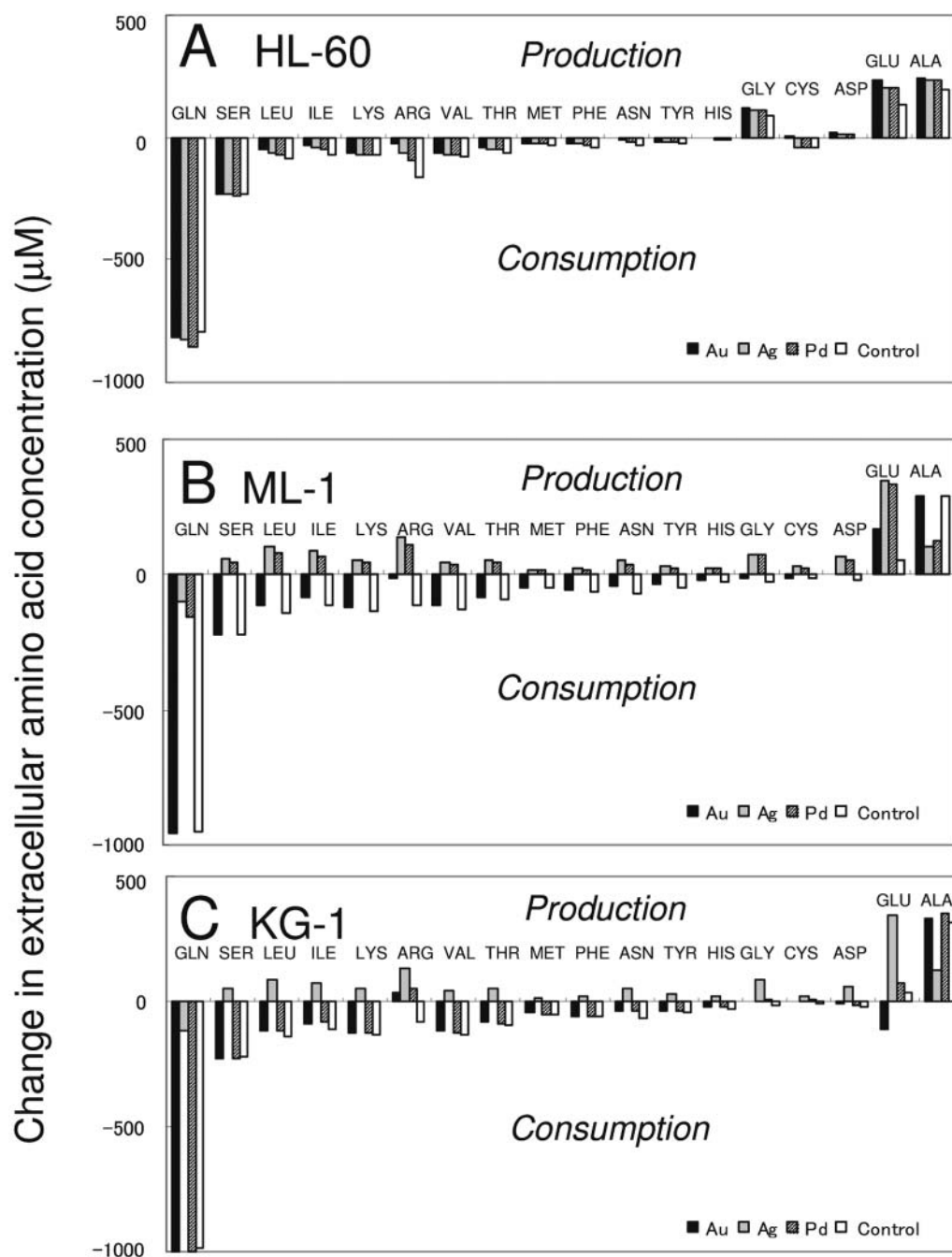


Figure 2. Effect of contact with metal plates on amino acid utilization. HL-60 (A), ML-1 (B) or KG-1 cells (C) were inoculated onto an Au, Ag or Pd plate, or plastic plate (control) and incubated for 0 or 24 hours. The TCA-soluble fraction of the cell culture medium was then applied to an amino acid analyzer. The ordinate represents the changes in the concentration of each amino acid in the culture medium during 24 hours' incubation.

GLN was reduced by 88.1% and the consumption of other amino acids was completely abrogated. On the other hand, the production of ARG, GLY and GLU was increased by 99, 89 and 234 nmole/mL/day, respectively, whereas the production of ALA was reduced by 207 nmole/mL/day.

Discussion

For the first time, we found that during the cell death of two human myelogenous leukemic cell lines (ML-1 and KG-1) induced by contact with an Ag or Pd plate, the

consumption of GLN, SER and most other amino acids was shut down, whereas production of GLY and GLU was very much enhanced. We recently reported that contact with Cu resulted in the rapid shutdown of the consumption of most amino acids, including GLN and SER, and an increase in the production of GLY and GLU in human gingival fibroblasts (10). Additionally, contact with Cu induced the enhancement of CYS consumption, possibly due to the oxidative action of Cu. These data suggest that metal-induced cell death is tightly coupled to the loss of the cell's ability to utilize GLN, SER and most other amino acids, and to produce GLY and GLU. GLN is a well known energy supplier (12), in addition to glucose. We previously reported that the growth of HL-60 depends on the extracellular SER concentration. When SER was left out of the culture medium, the growth of HL-60 cells immediately stopped; the supplementation of SER restored the growth (13). The consumption rate of SER was found to be very high, second to GLN, in all three human leukemic cell lines (HL-60, ML-1, KG-1). This indicates that the interruption of GLN and SER consumption might have a serious effect on cell survival. GLU is known to be involved in neuronal cell death. Depending upon the neuronal cell type, at least two mechanisms are involved in glutamate-induced apoptosis: a caspase-3-dependent pathway and a caspase-independent pathway involving calpain and mitochondrial apoptosis-inducing factor (AIF) (14). The elevation of GLU may thus trigger the cell death signaling pathway in human leukemic cell lines. GLY is known as an inhibitory neurotransmitter, acting *via* GLY receptor Cl^- channels (15). The elevation of GLY may affect the cellular function *via* such a receptor system or other mechanisms. Enhancement of ARG production by contact with metal plates may lead to the enhanced production of nitric oxide (NO), since ARG is a precursor of NO (16).

The present study suggests that the induction of cell death by contact with Ag and Pd depends on the surface smoothness of metal plates. When the surface smoothness is not appropriate, cell death may be induced. Further study is needed to investigate the relationship between the surface smoothness of metals and their ability to induce cell death. We found that the concentration of most amino acids was increased in the dying cells. It remains to be investigated whether this phenomenon is due to enhanced proteolysis in the autophagosome (17).

It has been reported that various toxicants, environmental hormones, antitumor agents and radiation affect cellular proliferation and functions in a bimodal fashion: a stimulatory (beneficial) effect at lower and a cytotoxic (adverse) effect at higher concentrations (strength) (18). We found that Au, and occasionally Ag and Pd, enhanced the cell growth of human leukemic cells. This may be the

beneficial part of hormesis induced by metals. The mechanism by which the stimulatory (beneficial) actions of metals are switched to detrimental actions remains to be elucidated.

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Received February 21, 2007

Revised May 7, 2007

Accepted May 21, 2007